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**EVALUATION OF THE AQUATIC TOXICITY AND
FATE OF BRASS DUST USING THE
STANDARD AQUATIC MICROCOSM**

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July 1988

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PREFACE

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EVALUATION OF THE AQUATIC TOXICITY AND FATE OF BRASS DUST
USING THE STANDARD AQUATIC MICROCOSM

1. INTRODUCTION

Studies on the acute toxicity of brass dust to daphnia and algae have been completed recently.^{1,2} In addition, the chemistry of the dissociation of the particles into copper and zinc ions has been the topic of recent reports. Although these assays have proven to be very useful in protecting a variety of species, no information was available for the impacts of toxicants on many aspects of the dynamics and metabolism of an ecosystem. A multispecies assay was sought to mimic the interactions and processes of a natural ecosystem.

In a properly designed system, interspecific interactions both within and between trophic levels can be analyzed. Parameters of ecosystem metabolism such as nutrient uptake, production, and respiration can be important clues to the long term impact of a material and should be included in the protocol. In addition, a multispecies assay should be able to provide data on the transformation and fate of the material in natural ecosystems.

Other, perhaps more practical criterion were also incorporated in the evaluation process and are similar to the criteria of Hammons.³ Generality was an important criteria because a generic freshwater assay was desired. The assay had to be well documented as to its behavior under the impact of at least one well-understood toxicant. Rejection standards or at least some first approximations of rejection standards had to exist so that a poor assay could be identified. Replication within an experiment had to be demonstrated. A standardized protocol that was sufficiently detailed for the preparation of a standard operating procedure (SOP) had to exist. Lastly, but equally important, when used to screen materials for long-term ecosystem-level effects, a multispecies assay had to be highly repeatable regardless of the year, laboratory, or geographical location.

Using the above criteria as a guide, we selected the Standard Aquatic Microcosm (SAM) developed by Dr. Taub,

University of Washington (Seattle, WA) as an ecosystem-level assay. The SAM protocol has undergone an extensive and lengthy period of research and development.^{4,5,6,7,8} During the last 3 years, we have participated in the FDA-supported round robin evaluation of the method using copper sulfate as the toxicant.

The SAM was a reliable and repeatable assay in the round robin testing.* A minor change in the protocol was made in the sterilization procedure to reduce the breakage of the test vessels during the experiment. Several minor changes made in the counting of organisms had no effect on the repeatability of the assays as determined by the round robin. With the beginning of the SAM, the amphipod stocks were also difficult to synchronize.

Brass dust produced signs of severe environmental and populational stress. Algal and daphnid populations demonstrated dose-response effects. Indices of community structure and metabolism exposed severe perturbations. The severe toxicity of the brass dust, predicted by the acute assays, was confirmed at the community level by the SAM methodology.

2. METHODS AND MATERIALS

2.1 Toxicant.

Brass dust was composed of 68.5% Cu, 27.5% Zn, with Al, Pb, Sb, palmitric acid, and stearic acid as minor constituents. The particles were an average diameter of 1.72 μ m and a thickness range of 800-320 nm.

2.2 Training.

Three members of the Ecological Toxicological Group (ETG) received training in the SAM protocol in the laboratory of Dr. Taub at the University of Washington (Seattle, WA) during a 3-day workshop. Subsequently, new employees were trained in-house. Dr. Taub's laboratory staff, especially Dr. Andrew Kindig, were unrelenting in their support of this research program.

*Taub, F.B., Kindig, A.C., Conquest, L.L., and Meador, J.P., American Society for Testing and Materials, Philadelphia, PA, 1988, unpublished data.

2.3 SAM Protocol.

The 64-day SAM protocol was described previously.⁹ Figure 1 shows the timetable of events. The microcosms were prepared by introducing 10 algal, 4 invertebrate, and 1 bacterial species into 3 L of sterile defined medium. Test containers were 4.0-L glass jars. An autoclaved sediment consisting of 200 g of silica sand and 0.5 g of ground chiten was autoclaved originally in the jar, but experience demonstrated that the resultant culture jars were very fragile, making the loss of a replicate very likely. Two different processes were tried, and both were effective in reducing the fragility of the vessels. First, the autoclaved sediment consisting of silica sand and ground chiten was added after the separate sterilizations of medium and vessel. Separate sterilization improved the durability of the glass jars and did not add to the contamination of the microcosms. Another modification of the process was to follow the SOP but to immerse the culture vessel in a water bath to a point above the sand and chiten level during sterilization.

Number of organisms, dissolved oxygen (DO), and pH were determined twice a week. Nutrients (nitrate, nitrite, ammonia, and phosphate) were sampled and measured twice a week for the first 4 weeks, and thereafter only once a week. Room temperature was 20 ± 2 °C. Illumination was $79.2 \mu\text{Em}^{-2}\text{sec}^{-1}$ PhAR with a range of 78.6-80.4 and a 16/8 day/night cycle.

A stock suspension of the brass dust was prepared for distribution to sample vessels. The material was weighted on a Cahn-28 electrobalance and dispensed into disposable polycarbonate tubes; diluent was added to make a 1-mg/mL suspension. The sealed tube was placed in an ultrasonic water bath and manipulated until all particles were as uniformly suspended as possible. Exposure concentrations were set so that an estimated no effect and an effect level could be expected from Daphnia magna 48-hr and algal 96-hr growth assay results.^{1,2} The brass concentrations were 0.01, 0.5, and 1.0 mg/L.

2.4 Chemistry.

The sampling and analytical methods have been previously reported.^{9*} Samples for dissolved metal analysis were collected once a week from each SAM vessel. The sample was

*Some information was found in unpublished data by Haley, M.V., Johnson, D.W., Muse, W.T., Jr., and Landis, W.G., 1987.

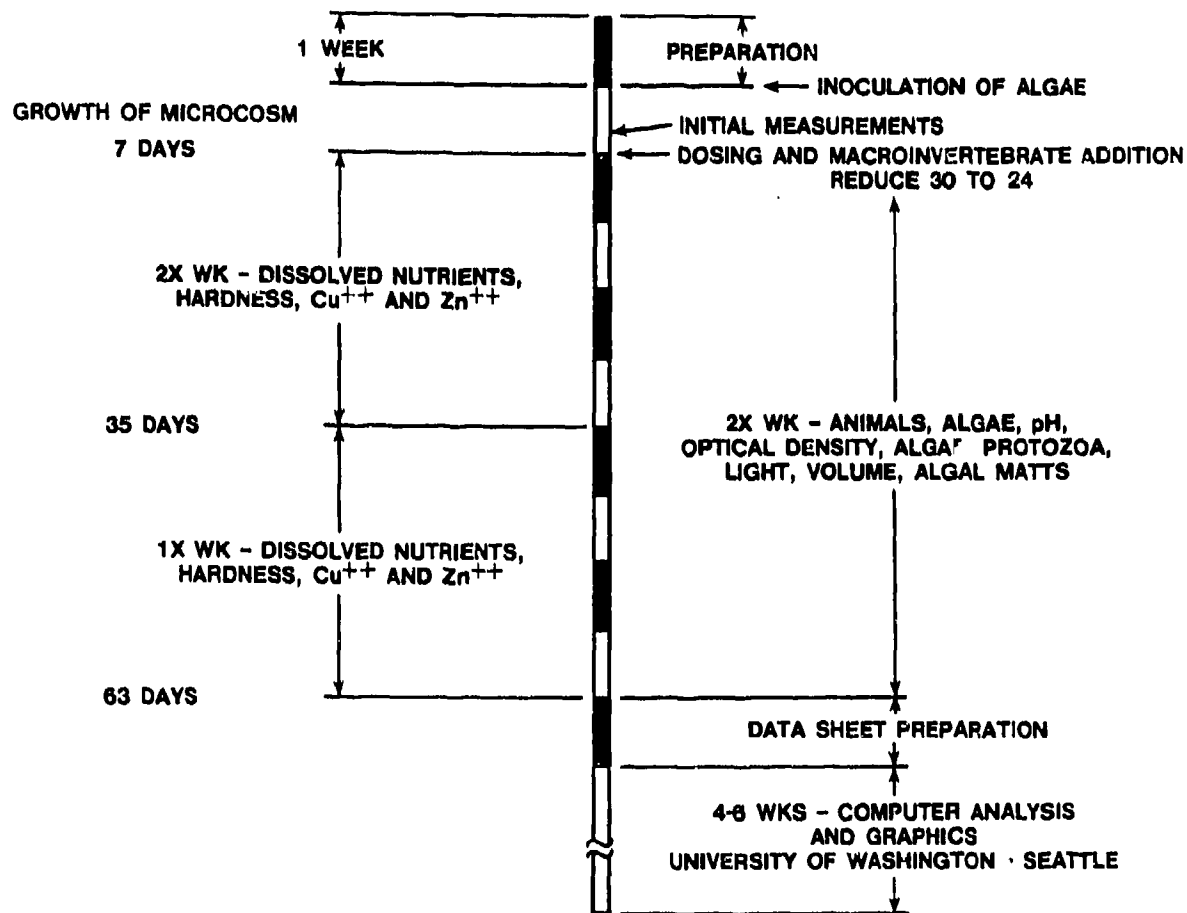


Figure 1. Timeline for the Standard Aquatic Microcosm (SAM).

passed through a Swinex 25-mm filter holder, and the sample was filtered with a prewashed 0.45- μ filter. Sample analysis for soluble copper was conducted on an atomic absorption spectrophotometer set at a wavelength of 324.7 nm, slit width 0.7 mm, air acetylene plume, and a lamp of copper [Hollow Cathode Lamp (HCL)] 18 ma. The analytical sensitivity was 0.03 mg/L copper.

2.5 Data Analysis.

All data were recorded on standard computer entry forms and checked for accuracy. The forms were then sent to the University of Washington for input and analysis. Parameters calculated included the concentrations each of DO, DO gain and loss, nutrient concentrations, net photosynthesis/respiration ratio (P/R), pH, algal species diversity, daphnid fecundity, algal biovolume, and biovolume of available algae. The statistical significance of each of these parameters compared to the controls was also computed for each sampling day.

3. RESULTS

3.1 Toxicant Fate.

The concentration of dissolved copper disassociated from the brass was monitored from microcosm day 7 to 63 (Figure 2). Analysis directly after the addition of brass showed that it did not disassociate immediately; dissolved copper remained at background levels. By day 14 (7 days post addition of brass), 23% of the copper had disassociated from the brass at both the medium and high dose levels. The dissolved copper concentration undergoes a slight decrease from day 21 to 28, then attains equilibrium from day 35 to 63. During equilibrium, both the medium and high dose levels remained at the same copper concentration. This represented a disassociation of at least 26% soluble copper at the medium dose level and 15% at the high dose level. Both low dose and control copper levels were below detectable limits, 0.03 mg/L, throughout the study.

3.2 Interspecific Interactions.

The interaction between the algae, daphnia, and toxicant concentration responded in a dose-response manner. In the controls, the *D. magna* reached a peak population density within 20-25 days and was followed by a regular decline. During this period, total algae stayed low with minor peaks due to

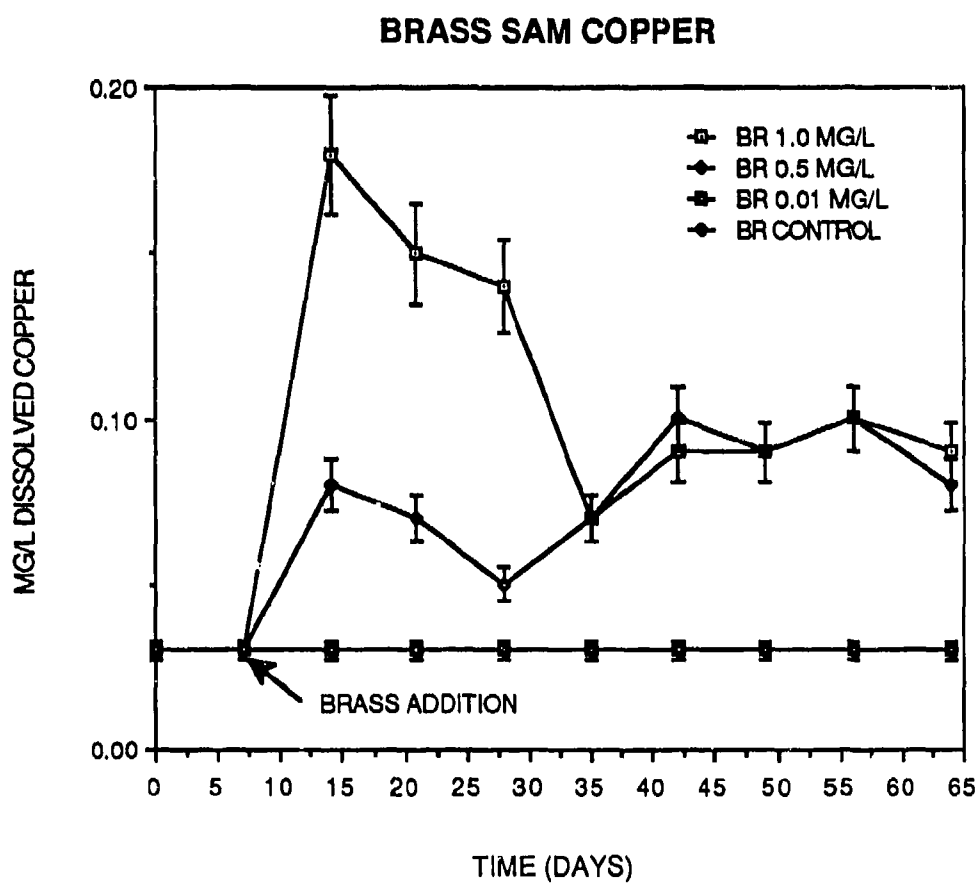


Figure 2. Concentration of Dissolved Cu in the SAM.

blooms by species less available to the daphnia (e.g., blue-greens). The density of algae and the population dynamics of the daphnia at a concentration of 0.01-mg/L brass were not significantly different from the controls (Figure 3a). As the toxicant concentration increased so did the peak algal density although the timing of the peak occurred later in the course of the experiment (Figure 3b). The key to the increase in the algal density that followed the toxicant density is the delay in the reproduction of the daphnid population with an increase in the toxicant. At the medium and high dose levels, the daphnia took 21 and 46 days respectively to demonstrate positive population growth. As the daphnid population grew, the algal population declined.

3.3 Species Diversity.

Algal species diversity (Shannon-Weaver) demonstrated the variability of this measure of community structure (Figure 4). The brass-SAM diversity in all of the dosage groups was superimposable for the first 17 days of the experiment. The medium dosage group, 0.50 mg/L, deviated markedly downward from all other dosage groups until midway through the experiment. At the end of the experiment, the diversity of the 0.5 mg/L group had recovered to the control values. In contrast, the group with highest toxicant concentration, 1.0 mg/L, tracked the control values until after day 40 of the experiment. At day 64, algal diversity in the highest dosage group was far below that of the other groups.

3.4 Nutrients.

Nutrients within the SAM demonstrated a dose-response relationship. For example, phosphate deviated significantly from the control values (Figure 5). In the brass-SAM, phosphate in the 1.0 mg/L replicates followed the declining trend of the other dosage groups but remained significantly higher until near the end of the experiment where an apparent equilibrium was reached. Nitrate (Figure 6a) demonstrated that use of the material was significantly slower in the highest dosage group. Nitrite (Figure 6b) increased initially but declined during the course of the experiment. Again, the decrease was slowest in the highest dosage group. Ammonia (Figure 6c) reached its highest concentrations in the control and lowest dosage group. The medium and highest dosage groups were significantly different from the controls but the response did not appear to be strictly

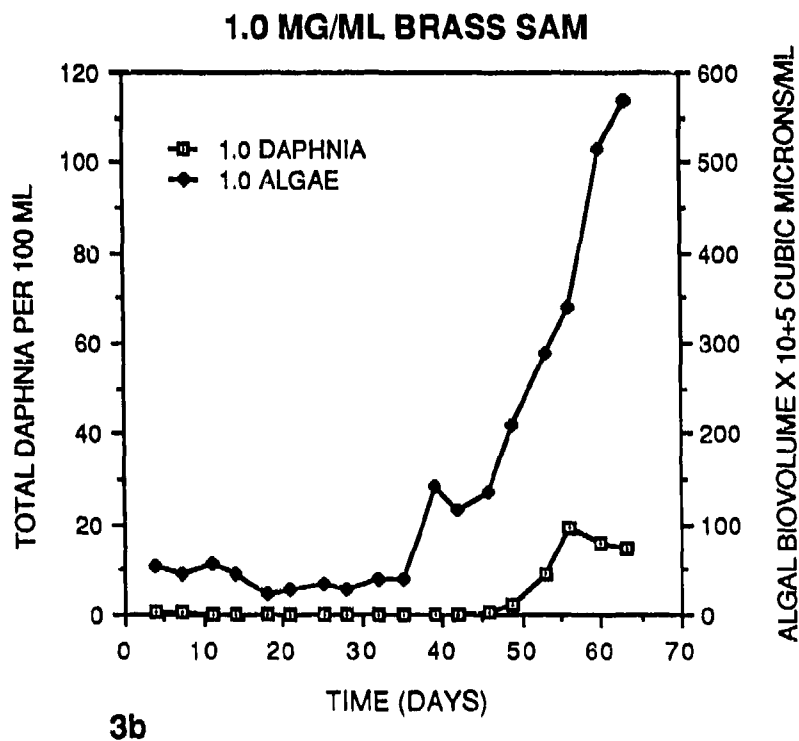
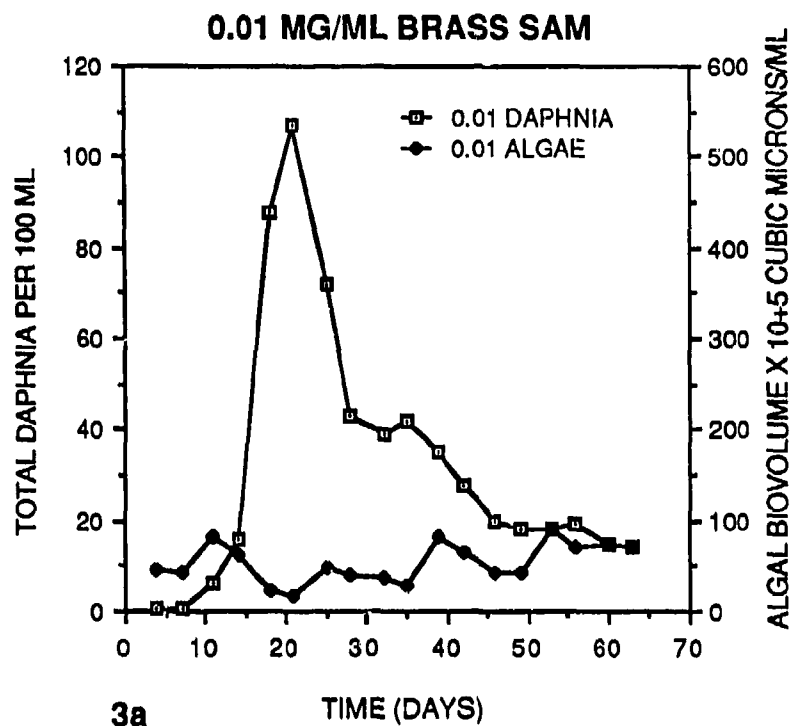


Figure 3. Comparison of Algal and Daphnid Growth Patterns in the SAMs.

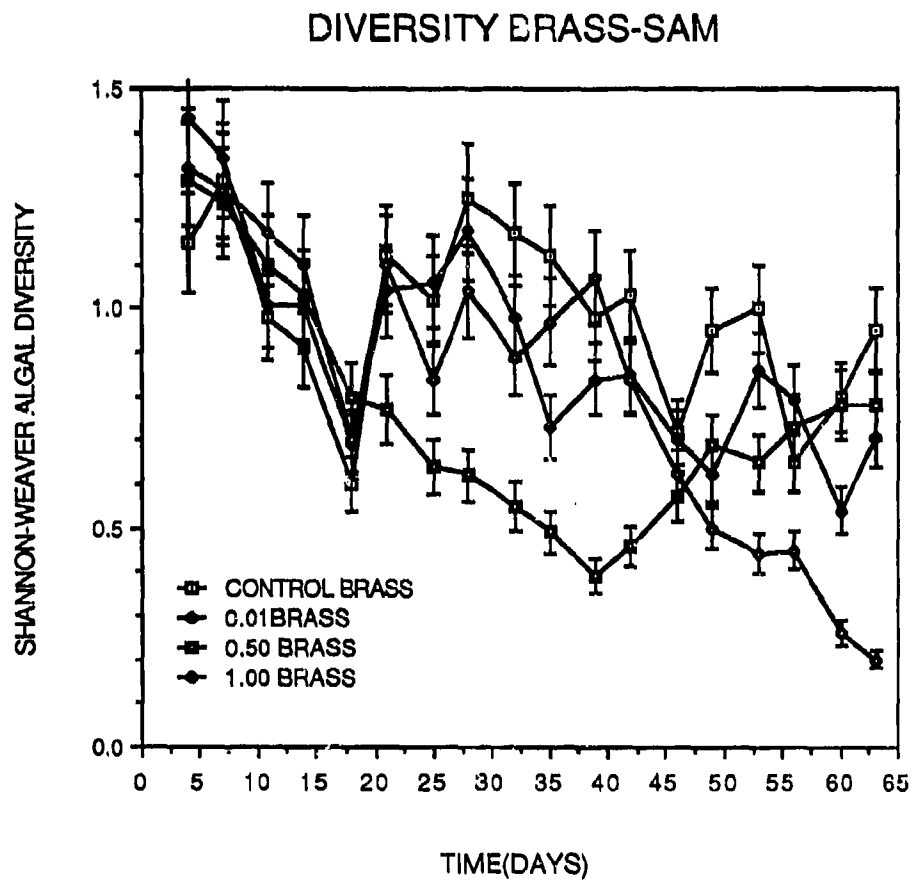


Figure 4. Species Diversity.

PHOSPHATE BRASS SAM

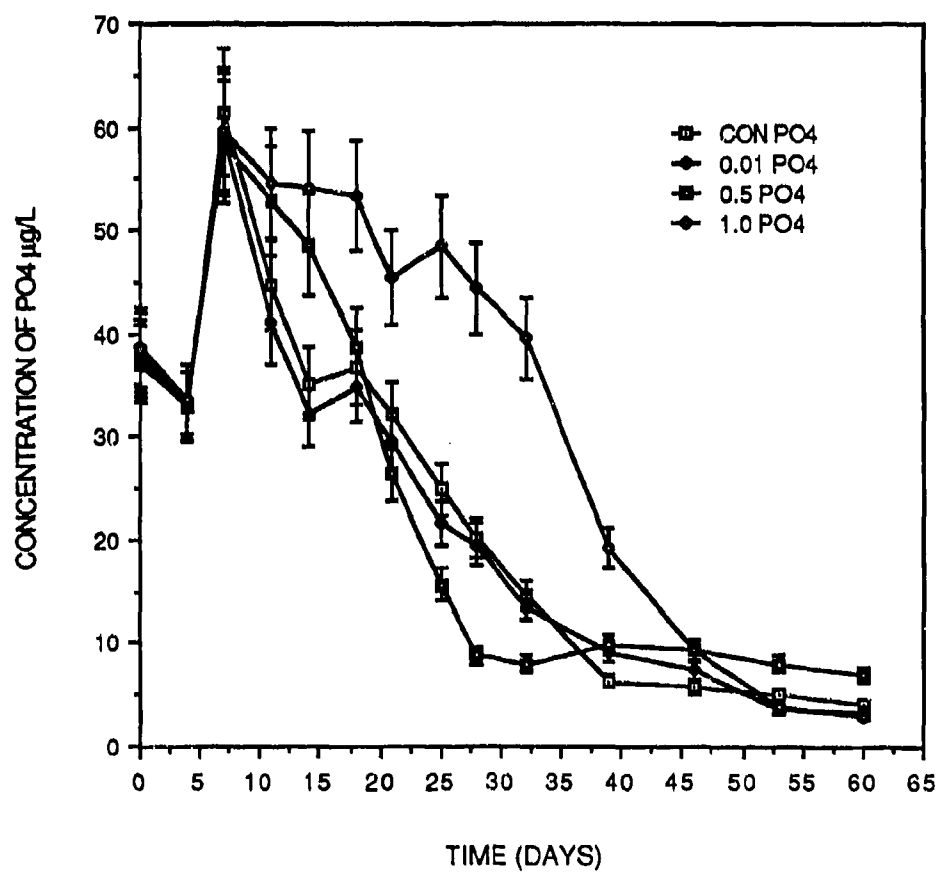
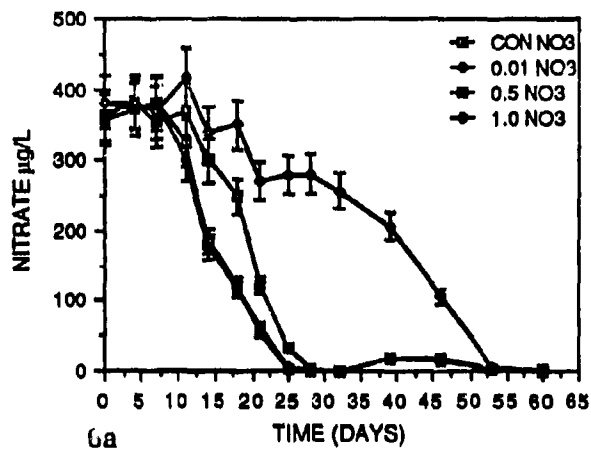
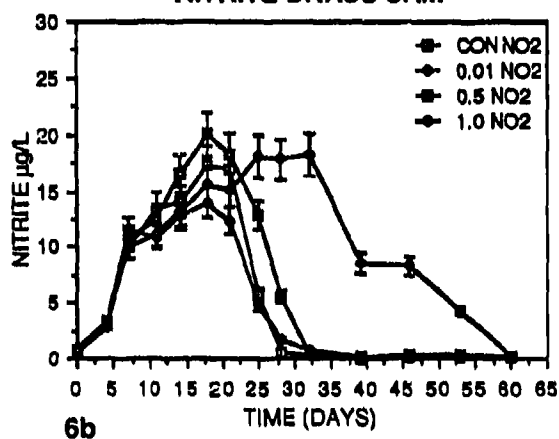


Figure 5. Phosphate Metabolism.

NITRATE BRASS SAM



NITRITE BRASS SAM



AMMONIA BRASS SAM

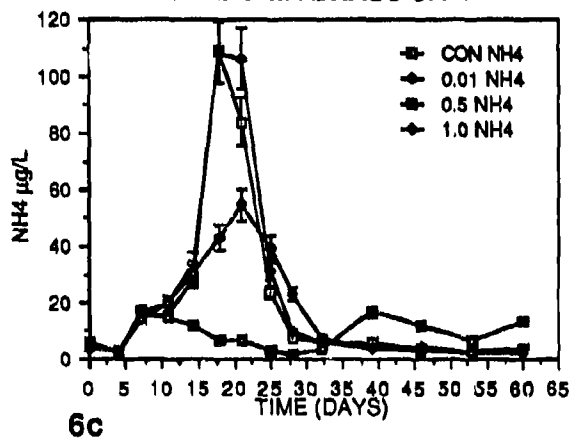


Figure 6. Nitrogen Cycling.

dose dependent. Silicate concentrations (Figure 7) fluctuated markedly, but a significant change due to the addition of a toxicant did not occur.

3.5 Photosynthesis/Respiration Ratio.

The photosynthesis/respiration ratio fluctuated and often ranged to below 1 in the highest concentrations of the brass-SAM (Figure 8). In the brass-SAM, all but the highest concentrations tracked the control replicates well. In the highest concentration, the P/R ratio appeared unstable as it dropped below 1 three times before returning to the equilibrium ratio.

4. DISCUSSION

The SAM appeared to be capable of exhibiting a variety of effects due to the application of the toxicant. The brass-SAM demonstrated the differential toxicity of the toxicant to the daphnid population and algae. The proportional increase in biomass of the algae to the concentration of the toxicant was a direct outcome of the differential toxicity. During sampling, resuspension of the brass also made the toxicant repeatably available to the filter feeding organisms; but, in temperate lakes, the fall and spring turnovers mix bottom material with the water column. Nutrient cycling in the brass-SAM also demonstrated stress in a dose-response manner. The nitrogen and phosphate cycling were clear examples.

In evaluating toxic effects, individual criteria such as species diversity, biomass, P/R ratio, and nutrient cycling cannot be used individually to identify an impact. For example, ecosystems that are undergoing stress are forced back to earlier successional stages, and therefore, species diversity drops. In the brass microcosm, only the highest concentration demonstrated a decrease in algal diversity and then only after the halfway point of the experiment. Although the 0.5 mg/L concentration demonstrated effects in algal and daphnid growth attributable to the brass, no significant decrease in the algal diversity was apparent. Two explanations come to mind. First, the SAM protocol is examining the system during its early development and maturation. Kindig and co-workers¹⁰ have previously shown that mature microcosms, without excess nutrients, are more susceptible to environmental stress. However, the use of developing microcosms is justified in that many aquatic systems undergo seasonal development with a succession of biota depending on temperature, light, and colonization. Secondly, the idea that a

SILICATE BRASS SAM

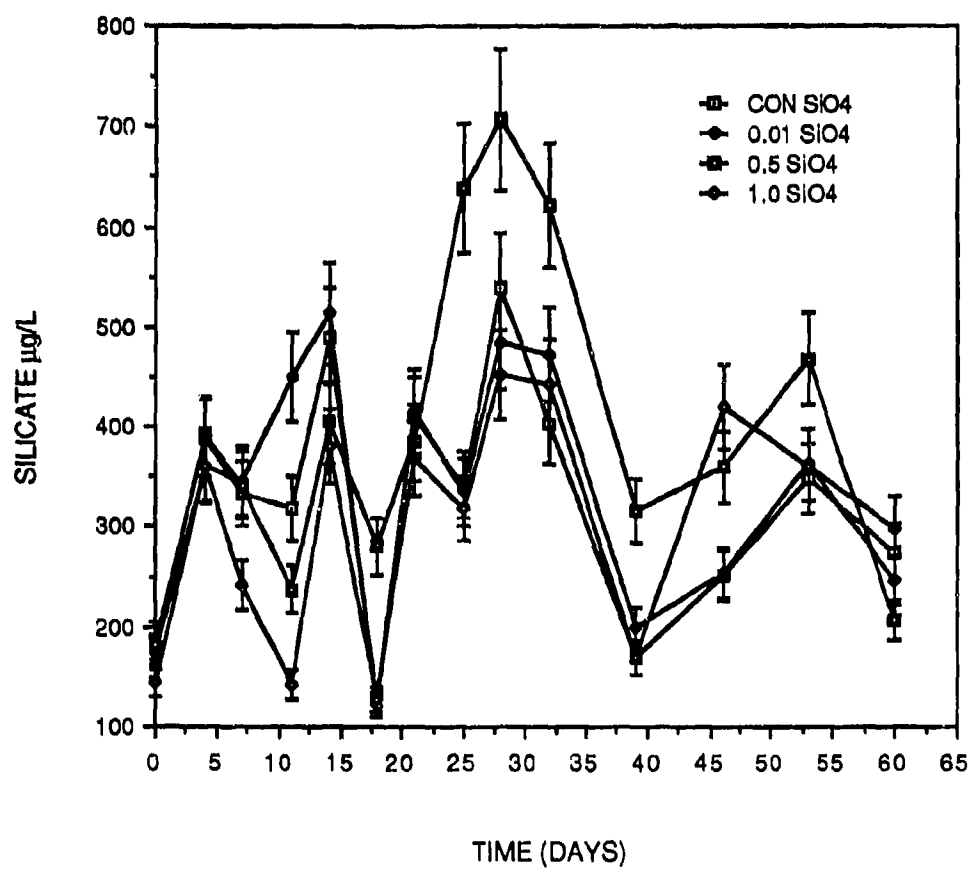


Figure 7. Silicate.

P/R RATIO BRASS-SAM

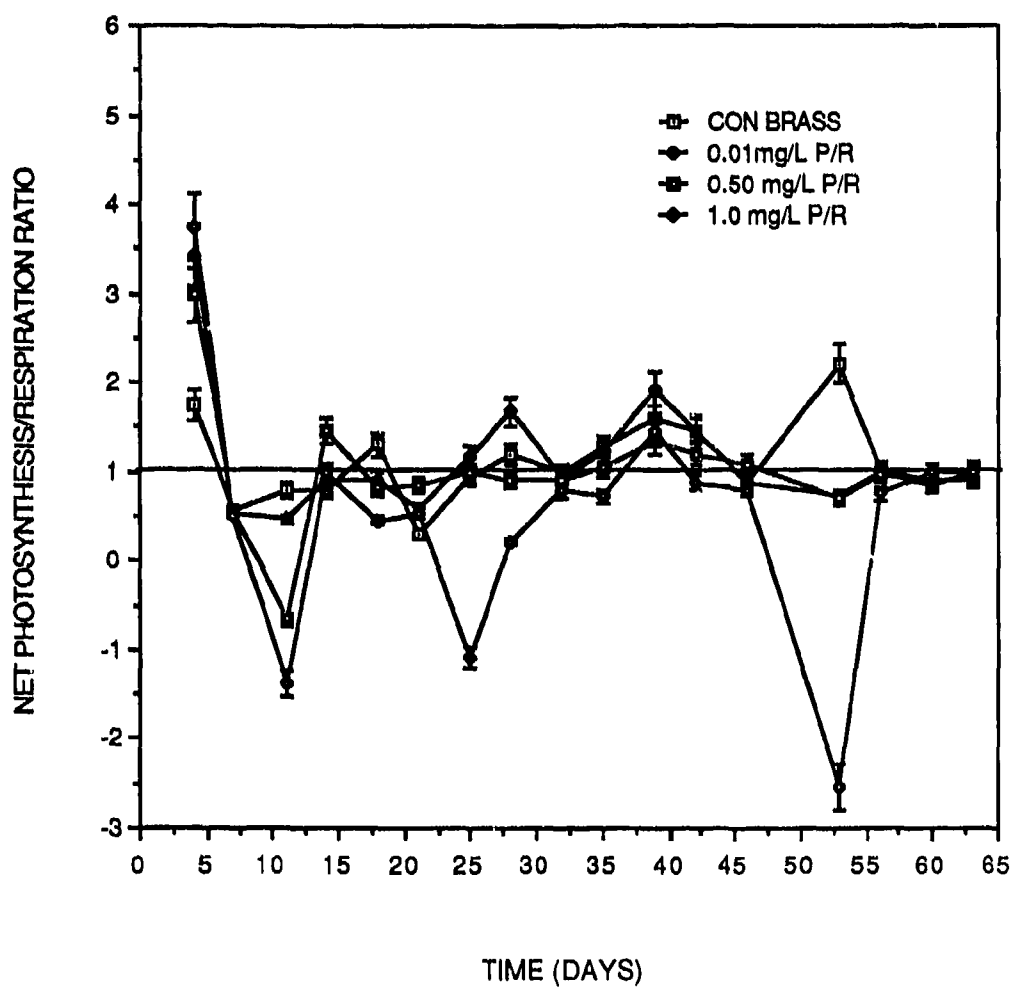


Figure 8. Photosynthesis/Respiration Ratio (P/R).

toxic effect automatically manifests itself in a decrease in diversity is an erroneous assumption. Landis,¹¹ using derivations of the resource competition models of Tilman,¹² has demonstrated that a toxicant can increase species diversity in the two species case. The brass-SAM illustrates the insensitivity of diversity as an indicator of toxicant stress.

Fate of the toxicant can be followed; the results mimic those of a natural ecosystem as opposed to a laboratory abiotic experiment. In laboratory experiments using waters of varying hardness, the brass disassociated into copper and zinc (unpublished data by Muse, 1987). The disassociation occurred whether the material was placed on the surface of the water or sonicated into suspension. The concentrations of these materials increased over time. For the brass-SAM, the concentration of copper decreased after an initial period following disassociation. Copper concentration reached an equilibrium in a fashion very similar to the copper in the CuSO_4 SAMs run as part of the interlaboratory round robin. Complexation of metals due to biologically derived chelates and binding to substrates has been extensively documented.^{13,14,15} Copper can also be eliminated due to the formation of insoluble precipitates in the presence of carbonate.¹⁶ In the fate of the copper at least, the SAM mimicked larger ecosystems.

The importance of understanding interactions among populations is crucial in estimating the long-term impact of the introduction of an xenobiotic. In the algal population of the SAM, a reversal of the expected dose response relationship was observed. The greater sensitivity of the daphnia allowed the algae to escape and reach higher population densities at higher toxicant concentrations. A toxicant can impact many levels of an ecosystem from the replication of the DNA to the interactions of the communities' trophic levels. The severest shortcoming of a single species assay is that other than toxicity or fate directed by the species under examination, all other levels of community interaction are bypassed. These interactions not only include the classical predator-prey competition, but the toxicant may be directly affected by the variety of extracellular materials produced by an ecosystem and progressive transformation of the material during its metabolism by various species. At our present level of understanding, we are unable to predict community interactions on the basis of only acute assays.

As useful as multispecies assays such as the SAM may be, they certainly cannot mimic every aspect of an aquatic ecosystem. Scale effects are crucial. The SAM, because of its

homogeneity of habitat and size, would be devastated by a large predator such as fish. Unless the laws of physics can be altered, the turbulent mixing, boundary layer, and stratification found in many aquatic ecosystems cannot be adequately simulated on the scale of a typical microcosm. Attempts to do so can be misleading.

Although there are limitations to the ability of the SAM to mimic full-scale ecosystems, a brass concentration as low as 0.5 mg/L (0.5 ppm) had long-term effects. A brass concentration of 1 mg/L (1 ppm) affected the growth of the daphnid population over a 50-day period. In many temperate zone ecosystems, this would correspond to a large portion of the growth season for cladocerans. Significant alterations in community metabolism were also noted. The literature cites many cases where heavy metal toxicity is heightened or ameliorated by specific conditions. A pH below neutrality has been known to increase copper toxicity significantly.¹⁷ The ability of many anions to complex copper and reduce toxicity is well known.¹⁸ However, the presence of heavy metals in low concentrations has consistently adversely affected the functioning of aquatic ecosystems.

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